SURVIVAL AND GROWTH OF LARVAE OF THE EUROPEAN OYSTER (*OSTREA EDULIS* L.) AT DIFFERENT TEMPERATURES

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A small number of European oysters (*Ostrea edulis*) was imported into the United States in 1949 for a study of the adaptability of this species to our waters. It was hoped that this oyster might be suitable for colder areas because in its northern range the European oyster reproduces at temperatures too low for the American oyster (*Crassostrea virginica*) to propagate (Loosanoff, 1955; Loosanoff and Davis, 1963). Some of these oysters were kept at Milford where we have reared a new generation almost every year to replenish our stock and to supply seed to other areas for studies of survival and growth. Others were planted in several estuarine areas of Maine; they reproduced naturally and have become established in Boothbay Harbor (Loosanoff, 1955). Seed oysters reared at Milford have been shipped to interested state shellfish biologists in California, Washington, and Alaska, but at present we know of no area in these states where a population has become established. Several single individuals have been found, however, attached to dead shells of Japanese oysters in Tomales Bay, California, where large numbers of Milford-reared *O. edulis* have been used in transplanting experiments (Loosanoff, personal communication).

In recent years shellfish hatcheries have been used more and more to propagate desirable commercial mollusks in areas where they do not propagate naturally or where naturally produced seed is insufficient. Successful operation of these hatcheries depends upon an adequate knowledge of the spawning habits of the mollusks and of the environmental requirements of their larvae.

Walne (1956, 1963, 1964, and 1965) studied several aspects of the rearing of larvae of *O. edulis,* especially the food requirements, and Davis and Ansell (1962) studied the salinity tolerance of these larvae. Korringa (1941) reviewed many field studies in an attempt to correlate temperature with duration of the larval period of *O. edulis* in European waters, and he believed that the length of the free-swimming period depended primarily on temperature. Walne (1965) reported on the influence of food supply and temperature on the growth of *O. edulis* larvae, but did not suggest an optimum temperature nor an upper limit of the temperature range for growth and survival. He suggested a "biological zero temperature of 13° C" (p. 30) at which no growth should occur. From his determinations of 24-hour growth rates he concluded that, "In the pelagic phase the time taken to grow from 175–250 μ decreases from 14 days at 17° C to 5 days at 25° C" (p. 42). No previous work, however, has been done on the survival and rate of growth of these larvae from time of release to setting under controlled conditions at different

constant temperatures. In the present study we determined the temperature range within which the larvae of *O. edulis* can exist and the optimum temperature for their growth.

METHODS

A series of 11 experiments was conducted in which duplicate 1-liter cultures of larvae were grown at each of six different temperatures in each experiment.
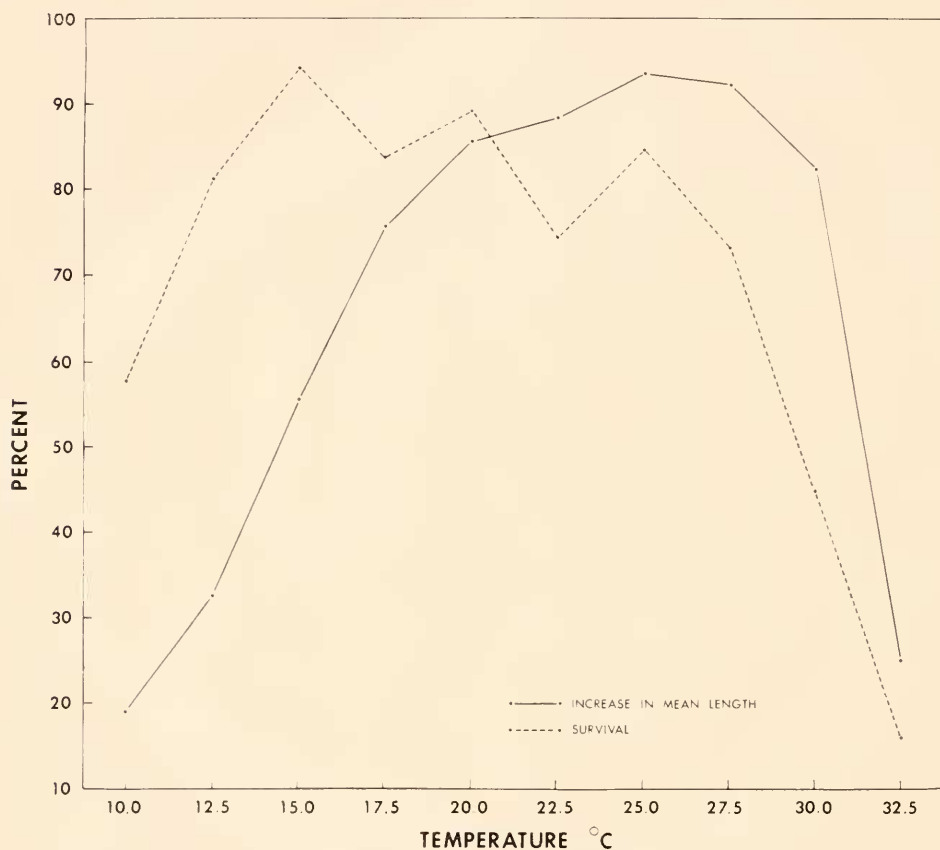

FIGURE 1. Survival and growth of larvae of *O. edulis* at different temperatures. Growth is expressed as average percentage increase in mean length for 11 experiments (Table I). Survival is average percentage survival in 11 experiments (Table II).

Since our apparatus can maintain only six different constant temperatures at a time, it was necessary to use a different series of temperatures in different experiments to cover the range from 10° to 32.5° C in 2.5° C steps. We considered it desirable to repeat certain reference temperatures in all experiments to facilitate comparisons among experiments. The temperatures of 20° and 27.5° C, therefore, were included in all experiments.

An accurately determined number, usually between 6000 to 8000, of recently released larvae was placed in each 1-liter polypropylene beaker containing filtered, ultraviolet-treated sea water (salinity 27 ± 0.5 ppt). All larvae were fed daily with a mixture of *Monochrysis lutheri, Dicrateria* sp. BII, and *Chlorella* sp. (Indiana University Collection #580). The sea water in each culture was changed every second day to eliminate waste products of larval metabolism. In most experiments 33 mg/l of Sulmet were added to each culture with each change of water to control bacteria which were troublesome at the higher temperatures. [Sulmet, sodium sulfamethazine, is a trade name of American Cyanamid Co. Mention of trade names does not imply endorsement of the product by the Bureau of Commercial Fisheries.]

TABLE I

Growth of O. edulis *larvae at different temperatures**

Expt. no.	Temperature (°C)									
	10.0	12.5	15.0	17.5	20.0	22.5	25.0	27.5	30.0	32.5
1	36.5	53.9	—	78.6	100.0	—	—	96.2	—	—
2	21.6	39.6	—	74.6	89.7	—	—	100.0	—	—
3	1.8	14.1	46.4	89.4	100.0	—	—	38.4	—	—
4	16.1	39.5	64.8	95.0	100.0	—	—	91.0	—	—
5	—	—	—	—	79.4	95.6	99.7	100.0	97.1	32.5
6	—	—	—	—	85.3	90.4	100.0	89.9	73.8	17.6
7	—	33.3	—	—	62.6	78.6	89.1	100.0	76.6	—
8	—	40.6	76.9	82.3	86.1	—	96.5	100.0	—	—
9	—	19.6	44.9	63.1	80.3	—	89.4	100.0	—	—
10	—	39.4	61.1	65.4	78.8	—	94.7	100.0	—	—
11	—	13.4	38.5	57.7	81.5	—	86.1	100.0	—	—
Average	19.0	32.6	55.4	75.8	85.8	88.2	93.6	92.3	82.5	25.1

* Increase in mean length is given as percentage of greatest increase at any temperature within that experiment. Figures are averages for duplicate cultures at each temperature in each experiment.

To determine the percentage survival and increase in mean length, the experiments were terminated after 8 or 10 days at the experimental conditions. Larvae in those cultures at near optimum temperatures were setting between the 10th and 12th days and satisfactory samples could no longer be obtained because once setting begins, not all of the larvae can be collected on the screens and resuspended. The larvae from each beaker were transferred to 250 ml of sea water in a graduated cylinder. The contents of the cylinder were thoroughly stirred to insure uniform distribution of the suspended larvae and a 4-ml quantitative sample (1.6% of the total population) was withdrawn and preserved with formalin. We examined each sample under a compound microscope, determined the number of larvae that survived the treatment and measured a random group of 50 individuals. Unpublished data indicate that this sampling technique yields survival figures accurate only to about $\pm 10\%$.

No one set of conditions can be considered "control" conditions in these experiments; therefore, survival at each temperature is expressed as a percentage of the number of larvae surviving in the pair of cultures, at a single temperature, that had the highest survival within that experiment. Increase in mean length is likewise expressed as a percentage of that of larvae in paired cultures showing the greatest increase. Since temperatures of 20° and 27.5° C were employed in each experiment, a comparison of percentages in successive experiments seems justifiable. All larvae were from *O. edulis* stock maintained at Milford except those used in Experiment 11. For this experiment we used larvae of *O. edulis* from the group that had become established in Boothbay Harbor, Maine.

TABLE II

Survival of O. edulis *larvae at different temperatures**

Expt. no.	Temperature (°C)									
	10.0	12.5	15.0	17.5	20.0	22.5	25.0	27.5	30.0	32.5
1	66.0	60.9	—	100.0	71.0	—	—	81.2	—	—
2	67.3	85.6	—	87.5	100.0	—	—	67.3	—	—
3	44.6	75.0	100.0	54.5	91.0	—	—	49.0	—	—
4	53.0	87.6	91.2	100.0	96.9	—	—	2.8	—	—
5	—	—	—	—	96.0	79.6	100.0	77.8	71.9	23.4
6	—	—	—	—	85.4	74.0	87.4	100.0	1.6	8.5
7	—	100.0	—	—	65.2	69.7	64.8	72.2	61.1	—
8	—	90.0	93.5	100.0	94.4	—	92.9	96.3	—	—
9	—	54.3	95.7	71.5	100.0	—	68.0	73.4	—	—
10	—	89.5	91.0	84.5	91.4	—	100.0	85.2	—	—
11	—	88.4	94.1	71.5	90.4	—	80.5	100.0	—	—
Average	57.7	81.2	94.3	83.7	89.2	74.4	84.8	73.2	44.9	16.0

* Survival is given as percentage of number of larvae surviving in the best culture within that experiment. Figures are averages for duplicate cultures at each temperature in each experiment.

Effect of Temperature on Growth

The average rate of growth of *O. edulis* larvae increased progressively as the temperature increased from 10° to 25° or 27.5° C and then decreased at 30° and 32.5° C (Fig. 1 and Table I). Although some growth did occur at 10° and 12.5° C and some larvae reached setting size at 12.5° and 15° C, we obtained no spat in these experiments at temperatures below 17.5° C. Growth was satisfactory (70% or more of maximum) only within the range from 17.5° to 30° C; at temperatures above and below this range, growth was too slow to be practical for shellfish culture. In nature a prolonged larval period would cause excessive losses due to predation and dispersal and in hatcheries it would greatly increase the cost of labor and make for inefficient use of equipment.

No one temperature consistently gave the most rapid growth of the larvae, although 27.5° C was optimum for growth in 7 of the 11 experiments (Table I). Moreover, if we disregard experiments in which survival was slightly less than 50% at 27.5° C (Table II), the average increase in mean length at 27.5° C be-

comes 96.8%, higher than the average for any other temperature. From examination of the living cultures, also, it was clear that the larvae grew best at 27.5° C. In some experiments, however, the proliferation of toxin-producing bacteria (or, as in Experiment 4, what appeared to be pathogenic bacteria) at 27.5° C and higher temperatures caused slow growth and poor survival.

Effect of Temperature on Survival

Survival was much more erratic than growth (Fig. 1 and Table II). Except for cultures kept at 10° C and those at 30° and 32.5° C, the average survival at each temperature was within the acceptable range (70% or more of optimum). Even at 10° and 30° C survival was poor, perhaps, largely because these unfavorable temperatures weakened the larvae, thus leaving them more susceptible to bacterial toxins and diseases. Increased bacterial populations, particularly noticeable at 30° and 32.5° C, could not be controlled by Sulmet. Nevertheless, the greatly reduced rate of growth at 32.5° C suggests that this temperature affected these larvae directly.

The excellent survival at 27.5° C in Experiments 6, 8, and 11 (Table II) confirmed our impression that poor survival at this temperature in other experiments was not the direct effect of temperature on the larvae but rather an indirect effect of the rapid proliferation of bacteria, which could not be adequately controlled by Sulmet. In many of the experiments the mortality at temperatures of 27.5° and 30° C occurred after the larvae were almost at setting size. In no experiment did we get as many larvae to set at 27.5° C as we did at somewhat lower temperatures. In Experiments 5 and 6, for example, starting with the same number of larvae at each temperature we obtained a total of 4964 spat at 20° C, 4863 at 22.5° C, 3709 at 25° C, 602 at 27.5° C, and only 14 at 30° C.

Setting times at each temperature varied considerably in these experiments because of variations in the quality of food cultures. Walne (1965) also reported that the rate of growth of *O. edulis* larvae at any given temperature varied in proportion to the quantity of food supplied, as well as the kind of algae used as food. In these experiments approximate setting times were as follows: 17.5° C—26 days, 20° C—14 days, and at 25°, 27.5°, and 30° C beginning of setting varied from the 8th to the 12th days.

Survival and Growth of Spat

A single experiment was conducted to determine the survival and rate of growth of *O. edulis* spat at different temperatures. Spat that had set less than 48 hours previously were placed at 10°, 12.5°, 15°, 17.5°, 20°, and 27.5° C. Survival was good at all temperatures except 10° C where growth was not appreciable; the spat still averaged only 0.3 mm in diameter after 30 days. Spat kept at 12.5°, 15°, 17.5°, 20°, and 27.5° C for 30 days averaged 0.5, 0.7, 1.5, 1.9, and 2.5 mm, respectively.

Evaluation and Recommendations for Culture

As shown previously, the maximum and minimum temperatures for growth of bivalve larvae depend to a large degree on the type of food provided and on salinity

(Davis and Calabrese, 1964). Although we believe the mixture of flagellates and *Chlorella sp.* (580) used as food in these experiments probably suffices at both temperature extremes, we cannot preclude the possibility that with other foods *O. edulis* larvae might grow satisfactorily at even lower or higher temperatures than are indicated in these experiments. Salinity (approximately 27‰) was about optimum (Davis and Ansell, 1962).

The temperature range for satisfactory growth of *O. edulis* larvae (17.5° to 30° C) is further evidence of the adaptation of this species of oysters to lower temperatures than *C. virginica,* since larvae of the latter species grow satisfactorily under our experimental conditions only at temperatures above 22.5° C (Davis and Calabrese, 1964). This difference in temperature tolerance of the larvae of *O. edulis* has been maintained even after approximately 15 generations grown at Milford. Moreover, the tolerance of larvae released by the stock of *O. edulis* that had become established at Boothbay Harbor, Maine (Experiment 11) did not differ from that of the larvae of Milford parents, although these two populations of *O. edulis* have been separated since 1949.

Walne (1965) reported that a brood of larvae reared at 14.3° C began setting on the 49th day (p. 29). Although 17.5° C was the lowest temperature at which we actually obtained setting in our experiments, larvae were grown to setting size at 12.5° and 15° C and we believe that setting could be obtained at 12.5° C if an experiment were continued long enough.

Walne (1965) stated that at all food-cell densities tested the uptake at 20° C is about 70% of that at the same cell density at 24° C (p. 34). Our data show that the average increase in mean length at 20° C is approximately 91% of that at 25° C. If we assume that the volume increases as the cube of the increase in mean length, then the increase in volume at 20° C is about 75% of that at 25° C or in reasonably close agreement with Walne's figure on food uptake.

It might be practical in hatchery operations to rear *O. edulis* larvae at 27.5° C until they reach a length of about 250 to 275 μ to take advantage of the more rapid growth and then reduce the temperature to 20° or 25° C to obtain a higher percentage of spat. Growth at 27.5° C, however, was not sufficiently faster than at 25° C to warrant the increased risk. Usually, it required only 1 or 2 days longer to obtain spat at 25° C than at 27.5° C and sometimes setting began simultaneously at both temperatures.

Summary

1. The temperature range for satisfactory growth of *O. edulis* larvae (70% or more of optimum) was from 17.5° to 30° C.

2. The temperature range for satisfactory survival (70% or more of optimum) was from 12.5° to 27.5° C. Even at 10° and 30° C survival was poor, perhaps, because the unfavorable temperatures weakened the larvae, making them more susceptible to bacterial toxins and diseases.

3. In these experiments approximate setting times were as follows: 17.5° C—26 days, 20° C—14 days, and at 25°, 27.5°, and 30° C beginning of setting varied from the 8th to the 12th days.

4. More spat were obtained at 20° to 22.5° C than at higher temperatures.

5. It is suggested that larvae be reared to setting size at temperatures from 25° to 27.5° C, then kept at 20° to 22.5° C during setting to obtain fastest growth of larvae and highest percentage setting.

6. Spat kept at 10° C showed virtually no growth; at temperatures from 12.5° to 27.5° C growth of spat increased with each increase in temperature.

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